1. INTRODUCTION:

1.1 Introduction to the research project:

Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the central nervous system. The project aims to develop and validate analytical methods for estimation of some anti-inflammatory drugs in bulk form and dosage forms. A major portion of the project involves analysis of thiocolchicoside and combined dosage forms of thiocolchicoside with some anti-inflammatory drugs. The developed methods were stability indicating methods which aid in estimation of drugs even in presence of degradation products. The methods were applied for analysis of drugs in marketed pharmaceutical dosage forms. Degradation kinetics of the thiocolchicoside in alkaline, acidic and oxidative conditions was carried out with the developed method.

1.2 Inflammation and Pain:

Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritant. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation is not a synonym for infection. Even in cases where inflammation is caused by infection, the two are not synonymous: infection is caused by an exogenous pathogen, while inflammation is the response of the organism to the pathogen.

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

Inflammatory disorders:

Abnormalities associated with inflammation comprise a large, unrelated group of disorders which underlie a vast variety of human diseases. The immune system is often involved with inflammatory disorders, demonstrated in both allergic reactions and some myopathies, with many immune system disorders resulting in abnormal inflammation. Non-immune diseases with a etiological origins in inflammatory processes are thought to include cancer, atherosclerosis, and ischemic heart disease.

A large variety of proteins are involved in inflammation, and any one of them is open to a genetic mutation which impairs or otherwise deregulates the normal function and expression of that protein.

Examples of disorders associated with inflammation include: asthma, autoimmune diseases chronic inflammation, chronic prostatitis, glomerulonephritis, hypersensitivities, inflammatory bowel diseases, pelvic inflammatory disease, reperfusion injury, rheumatoid arthritis, transplant rejection, vasculitis.

1.3 Anti-inflammatory drugs:

Anti-inflammatory drugs are the substances which are mainly used for treatment of inflammatory conditions and classified in two major classes.

Steroids:

Many steroids, specifically glucocorticoids, reduce inflammation or swelling by binding to cortisol receptors. These drugs are often referred to as corticosteroids.

Non-steroidal anti-inflammatory drugs:

Non-steroidal anti-inflammatory drugs, usually abbreviated to NSAIDs or NAIDs, are drugs with analgesic, antipyretic (lowering an elevated body temperature and relieving pain without impairing consciousness) and, in higher doses, with anti-inflammatory effects (reducing inflammation). The term "non-steroidal" is used to distinguish these drugs from steroids, which (among a broad range of other effects) have a similar eicosanoid-depressing, anti-inflammatory action. As analgesics, NSAIDs are unusual in that they are non-narcotic.

Classification of anti-inflammatory drugs:

Pyrazolidine / Butylpyrazolidines	Ampyrone · Clofezone · Kebuzone · Metamizole · Mofebutazone · Oxyphenbutazone · Phenazone · Phenylbutazone · Sulfinpyrazone
Acetic acid derivatives and related substances	**Aceclofenac** · Acemetacin · Alclofenac · Bromfenac · Bumadizone · Bufexamac · **Diclofenac** · Difenpiramide · Etodolac · Fentiazac · Indometacin · Ketorolac · Lonazolac · Oxametacin · Proglumetacin · Sulindac · Tolmetin · Zomepirac
Oxicams	Ampiroxicam · Droxicam · **Lornoxicam** · Meloxicam · Piroxicam · Tenoxicam
Propionic acid derivatives	Alminoprofen · Benoxaprofen · Dexibuprofen · Dexketoprofen · Fenbufen · Fenoprofen · Flunoxaprofen · Flurbiprofen · Ibuprofen · Ibuproxam · Indoprofen · Ketoprofen · Naproxen · Oxaprozin · Pirprofen · Suprofen · Tiaprofenic acid
Fenamates	Flufenamic acid · Meclofenamic acid · Mefenamic acid · Tolfenamic acid
Coxibs	Celecoxib · Etoricoxib · Lumiracoxib · Parecoxib · Rofecoxib · Valdecoxib
Others	Nabumetone · Niflumic acid · Azapropazone · Glucosamine · Benzydamine · Glycosaminoglycan · Magnesium salicylate · Proquazone · Superoxide dismutase/Orgotein · Nimesulide · Feprazone · Diacerein · Morniflumate · Tenidap · Oxaceprol · Chondroitin sulfate, **Thiocolchicoside**

1.4 Stability indicating method:

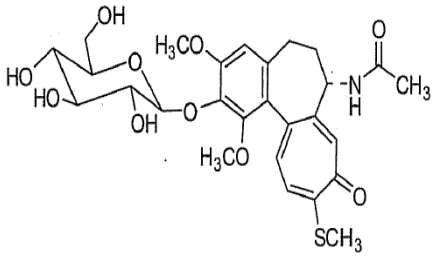
The accepted definition of a stability indicating method for a traditional (small molecules) pharmaceutical is a chromatographic (or other separation) method, able to separate the reportable degradants generated upon long-term storage of the product. Traditionally, the stability-indicating quality of the method is demonstrated by using stressed samples or long-term stability samples.

1.5 Degradation kinetic study:

Kinetic principles are of great importance in stability study of dosage form. The study of drug degradation kinetics is of greater importance for development of stable formulation and establishment of expiration date for commercially available drug products, in laboratories of pharmaceutical industries. In spite of the importance of degradation kinetic for development of stable dosage form, there have been relatively few attempts to evaluate the detail kinetic of their decomposition. The degradation rate kinetic gives the information regarding the rate of process that generally leads to the inactivation of drug through either decomposition or loss of drug by conversion to a less favorable physical or chemical form. The kinetic and stability are not identical but they are different in following ways, chemical kinetic is studies through half-lives. Stability studies down up to 85% of the initial strength. Chemical kinetic is carried out in pure system, while stability study system contains relatively many components. The goal of chemical kinetic is to elucidate reaction mechanism, where as that of stability study is to establish expiration date.

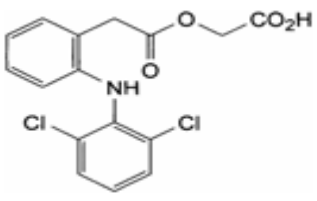
1.6 DRUG PROFILES:

Drug profile of Thiocolchicoside:

CAS NO	602-41-5
Pharmacopoeial Status	IP 2010
Category	Anti-inflammatory, Analgesic and Muscle relaxant
Chemical structure	
Empirical formula	$C_{27}H_{33}NO_{10}S$
Chemical Name	2-Demethoxy-2-glucosidoxythiocolchicine
IUPAC Name	N-[3-(ß-D-glucopyranosyloxy)-1,2-dimethoxy-10 (methyl thio)-9-oxo-5,6,7,9-tetrahydrobenzo[*a]heptalen-7-yl] acetamide*
Molecular weight	563.5gm/mol
Description	A yellow crystalline powder
Solubility	Soluble in Water & Alcohol
Melting point	218-220˚C
Pharmacological Profile	
Mechanism of action	It acts as a competitive GABA A receptor antagonist and also inhibits glycine receptors with similar potency and nicotinic acetylcholine receptors to a much lesser extent. Mode of action includes modulation of chemokine and prostanoid production and inhibition of neutrophil and endothelial cell adhesion molecules by which it interferes with the initiation and amplification of the joint inflammation.

Absorption	Thiocolchicoside is absorbed rapidly from the gastrointestinal tract, after oral administration and peak plasma concentrations are observed within approximately 1 h.
Distribution	Not clearly known
Metabolism	Thiocolchicoside is metabolized so rapidly after oral administration that it is impractical to determine concentrations in plasma over the periods generally used for bioequivalence studies. The aglycone, 3-desmethylthiocolchicine is the major metabolite and appears in plasma at concentrations which can be assayed over the required periods and therefore represents an appropriate surrogate analyte for the assessment of bioequivalence after oral administration of thiocolchicoside.
Excretion	Excreted by renal and nonrenal route.
Half life	4.5-5 hrs
Indication	For the treatment of backache, neuralgia, pain, parkinsonism, sciatic pain.
Storage condition	Protected from light and at a temperature not exceeding 30°C

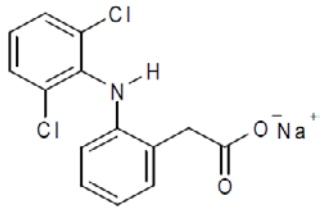
Drug profile of Aceclofenac:

CAS NO.	89796-99-6
Synonym	Aceclofenacum
Pharmacopoeial Status	IP 2010, BP 2010, EP 2011
Category	Anti-arthritic, Anti-inflammatory, Analgesic
Chemical Structure	
Empirical Formula	$C_{16}H_{13}Cl_2NO_4$

IUPAC Name	[[[2-[(2,6-Dichlorophenyl)amino]phenyl]acetyl]oxy]acetic acid
Molecular Weight	354.20 gm/mol
Description	White crystalline powder
Melting point	149-151^{0}C
Solubility	Practically insoluble in water, soluble in alcohol and methyl alcohol, freely soluble in acetone and di-methyl formamide
Partition Coefficient	3.03
Dissociation Constant (pKa)	4.7
Protein binding	> 99 % Plasma Protein bound
Half life	4 Hours
Pharmacological Profile	
Mechanism of Action	The anti-inflammatory effects of Aceclofenac have been shown in both acute and chronic inflammation. It inhibits various mediators of pain and inflammation including: • PGE2 via cyclooxygenase inhibition (COX-1 and COX-2) after intracellular metabolism to 4' –hydroxyl aceclofenac and diclofenac in human rheumatoid synovial cells and other inflammatory cells. • IL-1β, IL-6 and tumor necrosis factor in human osteoarthritic synovial cells and human articular chondrocytes.
Absorption	Aceclofenac is rapidly absorbed and the bioavailability is almost 100%. Peak plasma concentrations are reached approximately 1.25 to 3 hours
Distribution	Aceclofenac is highly protein-bound (> 99.7%). Aceclofenac penetrates into the synovial fluid where the concentrations reach approximately 60% of those in plasma.
Metabolism	Aceclofenac is probably metabolized via CYP2C9 to the main metabolite 4-hydroxyaceclofenac and other metabolites including 5-hydroxyaceclofenac, 4'-hydroxydiclofenac,

	diclofenac and 5-hydroxydiclofenac.
Excretion	Approximately two-thirds of the administered dose is excreted via the urine, mainly as conjugated hydroxyl metabolites and 20% is excreted in the faeces.
Half Life	4 Hours
Indication	Osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, dental pain, postoperative pain, dysmenorrhea, acute lumbago, musculoskeletal trauma, gonalgia (Knee pain).

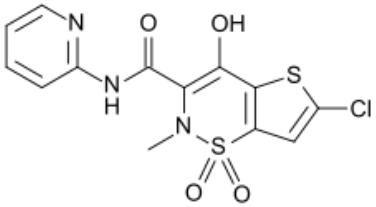
Drug profile of Diclofenac Sodium:

CAS NO	15307-86-5
Pharmacopoeial Status	British Pharmacopoeia 2010, Indian Pharmacopoeia 2010, European Pharmacopoeia 7.0.
Category	Diclofenac sodium is a cyclooxygenase Inhibitors and non-steroidal anti-inflammatory agent (NSAIA)
Chemical structure	
Empirical formula	$C_{14}H_{10}C_{l2}NNaO_2$
Chemical Name	2-(2, 6-dichloranilino) phenyl acetic acid.
IUPAC Name	2-[2,6dichlorophenylamino] benzene acetic acid sodium salt.
Molecular weight	318.1 gram/mole
Description	White or slightly yellowish crystalline powder.
Solubility	Sparingly soluble in water, freely soluble in methanol, soluble in ethanol, slightly soluble in acetone.
Melting point	283-285 °C
Pharmacological Profile	

Mechanism of action	Primary mechanism responsible for its anti-inflammatory, antipyretic, and analgesic action is thought to be inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX).
Absorption	Completely absorbed from GI track.
Distribution	Diclofenac is more than 99% bound to human serum proteins, primarily to albumin. Serum protein binding is constant over the concentration range (0.15-105 μg/mL) achieved with recommended doses.
Metabolism	Hepatic metabolism occurs with five diclofenac metabolites have been identified in human plasma and urine. The metabolites include 4'-hydroxy-, 5-hydroxy-, 3'-hydroxy-, 4',5-dihydroxy- and 3'hydroxy-4'-methoxy-diclofenac. The major diclofenac metabolite, 4'-hydroxy-diclofenac
Excretion	Diclofenac is eliminated through metabolism and subsequent urinary and biliary excretion of the glucoronide and sulphate conjugated of the metabolites.
Half life	2 hours
Indication	For the acute and chronic treatment of sign and symptoms of osteoarthritis and rheumatoid arthritis.

Drug profile of Lornoxicam:

CAS NO	70374-39-9
Pharmacopoeial Status	Not official in any pharmacopoeia
Category	Lornoxicam is a Non-steroidal anti-inflammatory drug (NSAID) of the oxicam class with analgesic, anti-inflammatory and antipyretic properties.

Chemical structure	
Empirical formula	$C_{13}H_{10}ClN_3O_4S_2$
Chemical Name	6-chlor-4-hydroxy-2-methyl-N-2-pyridyl-2H-thieno [2, 3-e]-1, 2 thiazine-3-carboxamide 1,1-dioxide.
IUPAC Name	(3*E*)-6-chloro-3-[hydroxy(pyridin-2-ylamino)methylene]-2-methyl-2,3-dihydro-4*H*-thieno[2,3-*e*][1,2]thiazin-4-one 1,1-dioxide
Molecular weight	371.82 gram/mole
Description	Orange to yellow crystals
Solubility	Slightly soluble in chloroform, Very slightly soluble in methanol and acetonitrile Insoluble soluble in water
Melting point	225-230°C
Partition coefficient	1.8
Pharmacological Profile	
Mechanism of action	Lornoxicam is anti-inflammatory and analgesic activity is related to its inhibitory action on prostaglandin and thromboxane synthesis through the inhibition of both COX-1 and COX-2.
Absorption	Lornoxicam is absorbed rapidly and almost completely from the GI tract (90-100%).
Distribution	The absolute bioavailability of Lornoxicam is 90–100%. No first-pass effect was observed
Metabolism	Lornoxicam is metabolized completely by CYP2C9 with the principal metabolite being 5'-hydroxy-lornoxicam and only negligible amounts of intact lornoxicam are excreted unchanged in the urine.
Excretion	The total excretion of lornoxicam via urine and faeces after oral

	administration was determined by administering 14C-labelled compound.
Half life	3-5 hours
Indication	Acute mild to moderate pain and inflammation of the joints.

2. LETERATURE REVIEW:

Table -1: Official method for analysis of THC in pure and pharmaceutical formulations

Sr. No.	Formulation	Description
IP-2010 Method		
1.	Thiocolchicoside API	**HPLC** **Stationary Phase:** Octadecylsilane packing of 5 µm on stainless steel column (25 cm x 4.6 mm) bonded to porous silica. **Mobile Phase:** **A :** Water **B :** Acetonitrile **A linier gradient programme is used** **Detection:** 370 nm **Injection Volume:** 20 µl
2.	Thiocolchicoside Capsule	Same as Thiocolchicoside API.

Table-2: Other reported methods for analysis of Thiocolchicoside

Sr. No.	Method	Description
Spectroscopic Methods		
	For only THC	
1.	UV Visible spectrophotometric	**Method A:** Maximum absorbance method

	determination of Thiocolchicoside in capsule	***λmax :*** 259.8 nm in water **Method B:** Area under Curve (AUC*)* **wavelength range:** 269.8-249.8 nm
2.	Spectrophotometric methods for the determination of Thiocolchicoside in bulk and pharmaceutical dosage forms	**Method A :** Zero derivative spectrum **λmax** :259.0 nm **Method B** :First derivative spectrum **λmax :**252.0 nm **Method C :**Second derivative spectrum **λmax :** 260.0 nm **Method D** : Area under Curve (AUC) **wavelength range**: 254.0 – 264.0 nm **Solvent**: 0.1 N NaOH
3.	UV Spectrophotometric determination of Thiocolchicoside from capsule dosage	The adequate drug solubility and maximum assay sensitivity was found in methanol. **λmax :** 257nm **wavelength range**: 200 - 400 nm
	<u>In combination with other Drugs</u>	
4.	Estimation of Thiocolchicoside and Ketoprofen in pharmaceutical dosage form by spectrophotometric methods	**Method A :** First order derivative spectroscopy **λmax** : For THC :233 nm For KET :243.0 nm **Method B** : Absorbance correction method **λmax** : 372 nm and 260 nm
5.	Simultaneous estimation of Etodolac and Thiocolchicoside by UV spectrophotometric methods in tablet formulations	Proposed method is based on the use of two wavelength that is λmax of THC 260 nm and wavelength of Etodolac 232 nm **Linearity:** 2 – 20 ppm and 15 – 100 ppm for THC and ETD, respectively. **Correlation Coefficient:** 0.993 and 0.9994 for THC and ETD, respectively
6.	Simultaneous estimation of Thiocolchicoside and Diclofenac	Multicomponent mode for detection. The detection was done using UV detector at 254,259,265,271,286

	potassium by UV spectrophotometer using multicomponent method	nm **Linearity:** 20-100ppm for both **Correlation Coefficient:** 0.998 for both
7.	Simultaneous UV-Spectrophotometric determination of Thiocolchicoside and Diclofenac in Pharmaceutical formulation	**Method A-** Simultaneous equation mehod **Detection:** THC at 260 nm and Diclofenac at 276.5 nm **Method B-** Absorbance correction Method Measurement at 373.0 nm (iso-absorptive point) and 276.5 nm (λ_{max} of Diclofenac). **Linearity:** 1-2 ppm and 6.25-62.5 ppm for THC and Diclofenac, respectively
8.	Spectrophotometric methods for simultaneous estimation of Etoricoxib and Thiocolchicoside in bulk and combined pharmaceutical dosage form	**Method A :** Simultaneous equation method **Method B** : Simultaneous equation method using AUC of the two drugs **Method C :** Absorption ratio method **Method D** : First order derivative method
9.	Simultaneous spectrophotometric estimation of Thiocolchicoside and Dexketoprofen in pharmaceutical dosage form	Dual wavelength spectrophotometric methods for simultaneous determination. Two wavelength selected are:368nm and 284.60 nm **Linearity:** 2-24 ppm for both **Correlation Coefficient:** 0.997 for both
10.	Development and validation of UV-Visible spectrophotometric methods for simultaneous estimation of Thiocolchicoside and Dexketoprofen in bulk and tablet dosage form	**Method A-** Absorbance correction Method **Detection:** Measurement at 255 nm (isoabsorptive point) and 370 nm (λ_{max} of THC). **Method B:** First order derivative spectroscopic method **λmax** : For THC :232 nm For Dexketoprofen :242.0 nm **Linearity:** 4-40 ppm and 5-50 ppm for THC and Dexketoprofen, respectively **Correlation Coefficient:** 0.997 for both drugs

11.	Development and validation of first order derivative spectroscopic estimation of Thiocolchicoside and Dexketoprofen Trometamol in pharmaceutical dosage form	In first order derivative spectroscopy, wavelengths selected for quantitation were 233.7 nm for Thiocolchicoside (zero cross for Dexketoprofen trometamol) and 243.0 nm for Dexketoprofen trometamol (zero cross for Thiocolchicoside). **Linearity:** 4-12 ppm for Thiocolchicoside and 25-75 ppm for Dexketoprofen trometamol
12.	Simultaneous estimation of Paracetamol, Thiocolchicoside and Aceclofenac by UV spectrophotometer using multicomponent method	Multicomponent method **Detection:** 249 nm, 258 nm and 276 nm for Paracetamol, Thiocolchicoside and Aceclofenac, respectively **Linearity:** 5-25 ppm, 1-5 ppm and 1-5 ppm for Paracetamol, Thiocolchicoside and Aceclofenac, respectively **Correlation Coefficient:** 0.999
13.	Novel spectrophotometric methods for simultaneous estimation and validation of Paracetamol, Thiocolchicoside and Aceclofenac in tablet dosage form by UV spectrophotometer	**Detection:** 256 nm, 258 nm and 270 nm for Paracetamol, Thiocolchicoside and Aceclofenac, respectively **Linearity:** 1-25 ppm, 2.5-25 ppm and 1-5 ppm for Paracetamol, Thiocolchicoside and Aceclofenac, respectively **Correlation Coefficient:** 0.999, 0.994, 0.995 for Paracetamol, Thiocolchicoside and Aceclofenac, respectively
Chromatographic Methods		
	For only THC	
14.	Stability indicating RP-HPLC methods for estimation of Thiocolchicoside in capsule dosage forms	**Stationary Phase**: C_{18} column (250mm x 4 mm, 5 µm). **Mobile Phase:** Acetonitrile : Water (70:30 v/v) **Flow rate:** 1.0 ml/min **UV Detection:** 286 nm

		Linearity: 0– 10 µg/ml **Correlation Coefficient:** 0.999 **Retention Time:** 3.345 min
15.	Development and validation of Stability indicating RP-HPLC and assay method for determination of Thiocolchicoside in capsule	**Stationary Phase**: Phenomenex ODS C_{18} column (250mm x 4.6 mm, 5 µm). **Mobile Phase:** Acetonitrile : Phosphate buffer (70:30 v/v) pH 3.5 is adjusted **Flow rate:** 1.0 ml/min **UV Detection:** 260.0 nm **Linearity:** 0– 10 µg/ml **Correlation Coefficient:** 0.999 **Retention Time:** 2.247 min.
	<u>In combination with other Drugs</u>	
16.	Simultaneous estimation of Etodolac and Thiocolchicoside in their combined marketed formulation by RP-HPLC	**Stationary Phase**: Phenomenex C_{18} column (250mm x 4.60 mm, 5 µm). **Mobile Phase:** Methanol : Phosphate buffer pH 6(85:15 v/v) **Flow rate:** 0.8 ml/min **UV Detection:** 259 nm **Linearity:** 16– 96 µg/ml **Correlation Coefficient:** 0.9993 and 0.9996 for THC and Etodolac, respectively **Retention Time:** 3.52 min and 4.39 min for THC and Etodolac, respectively
17.	Validated stability indicating RP-HPLC method for simultaneous determination and in vitro dissolution studied of Thiocolchicoside and Diclofenac Potassium from tablet dosage form	**Stationary Phase**: Zorbax SB CN (250mm x 4.60 mm, 5 µm). **Mobile Phase: Gradient Elution** 5mM Sodium Dihydrogen Phosphate buffer pH 2.5 :Methanol **Flow rate:** 1.0 ml/min **UV Detection:** 258 nm

		Retention Time: 5.850 min and 11.071 min for THC and Diclofenac, respectively
18.	Application of RP-HPLC method for simultaneous estimation of Thiocolchicoside and Diclofenac in commercially available capsules	**Stationary Phase**: Phenomenex Gemini C_{18} column (250mm x 4.60 mm, 5 µm). **Mobile Phase:** Acetonitrile: Water (70:30 v/v) $_{P}$H 3 is adjusted **Flow rate:** 1.0 ml/min **UV Detection:** 258nm **Linearity:** 4-24 ppm and 25-150 ppm for Thiocolchicoside and Diclofenac, respectively **Correlation Coefficient:** 0.9998 for both **Retention Time:** 1.537 min and 4.010 min for THC and Diclofenac, respectively
19.	RP-HPLC method for simultaneous estimation of Thiocolchicoside and Ketoprofen in combined dosage forms	**Stationary Phase**: Thermo scientific C_{18}, (250mm x 4.60 mm, 5 µm). **Mobile Phase:** Acetonitrile: Water : Phosphate buffer $_{P}$H 3 (60:30:10 v/ v/v) **Flow rate:** 1 ml/min **UV Detection:** 260 nm **Linearity: :** 4-20 ppm and 20-100 ppm for Thiocolchicoside and Ketoprofen, respectively **Correlation Coefficient:** 0.9950 and 0.9970 for THC and Ketoprofen, respectively **Retention Time:** 2.70 min and 4.90 min for THC and Ketoprofen, respectively
20.	Simultaneous estimation of Etoricoxib and Thiocolchicoside by HPLC method in combined dosage forms	**Stationary Phase**: BDS Hypersil C_{18} column, (250mm x 4.60 mm, 5 µm). **Mobile Phase:** Trifluoroacetic acid buffer ($_{P}$H 2.6): Acetonitrile(75:25 v/v) **Flow rate:** 1.5 ml/min **UV Detection:** 220 nm

		Linearity: : 2-16 ppm and 20-160 ppm for Thiocolchicoside and Etoricoxib, respectively **Correlation Coefficient:** 0.9994 and 0.9918 for THC and Etoricoxib, respectively **Retention Time:** 6.6 min and 3.1 min for THC and Etoricoxib, respectively
21.	Development and validation of RP-HPLC method for the simultaneous estimation of Thiocolchicoside and Dexketoprofen in bulk and pharmaceutical dosage forms	**Stationary Phase**: C_{18} column, (250mm x 4.60 mm, 5 µm). **Mobile Phase:** Methanol: Phosphate buffer (65:35 v/v) PH 4.5 is adjusted with Orthophosphoric acid **Flow rate:** 1.0 ml/min **UV Detection:** 260 nm **Linearity: :** 4-24 ppm and 5-30 ppm for Thiocolchicoside and Dexketoprofen, respectively **Correlation Coefficient:** 0.9998 and 0.9990 for THC and Dexketoprofen, respectively **Retention Time:** 3.02 min and 8.91 min for THC and Dexketoprofen, respectively
22.	Validated RP-HPLC method for simultaneous estimation of Lornoxicam and Thiocolchicoside in solid dosage form	**Stationary Phase**: Inertsil ODS 3V C_{18} column, (250mm x 4.60 mm, 5 µm). **Mobile Phase:** Methanol: Phosphate buffer (55:45 v/v) PH 7.3 is adjusted with **Flow rate:** 1.5 ml/min **UV Detection:** 290 nm **Linearity: :** 0.24-120 ppm and 0.235-120 ppm for Thiocolchicoside and Lornoxicam, respectively **Correlation Coefficient:** 0.9999 for both **Retention Time:** 2.96 min and 9.40min for THC and Lornoxicam, respectively
23.	Validated HPLC method for simultaneous estimation of	**Stationary Phase**: Hiq Sil C_{18} column, (250mm x 4.60 mm, 5 µm).

	Paracetamol, Aceclofenac, and Thiocolchicoside in bulk drug and formulation	**Mobile Phase:** Acetonitrile: Water (30:70 v/v) **Flow rate:** 1.0 ml/min **UV Detection:** 263 nm **Retention Time:** 2.52 min and 3.56 min and 5.20 min for Paracetamol, THC and Aceclofenac, respectively
24.	Development and validation of HPTLC method for simultaneous estimation of Lornoxicam and Thiocolchicoside in combined dosage form	**Stationary Phase:** Pre-coated silica gel G60 F_{254} aluminium plates. **Mobile Phase:** Methanol: Chloroform: Water (9.6:0.2:0.2 v/v/v). **Detection:** 377 nm **Linearity:** 60-360 ng/band and 30-180 ng/band for Lornoxicam and THC, respectively. **Correlation Coefficient:** 0.998 and 0.999 for Lornoxicam and THC, respectively. **R_f:** 0.84 and 0.58 for Lornoxicam and THC, respectively
25.	Validated HPTLC method for simultaneous estimation of Thiocolchicoside and Aceclofenac in bulk drug and formulation	**Stationary Phase:** Pre-coated silica gel G60 F_{254} aluminium plates. **Mobile Phase:** Toluene: Ethyl Acetate: Methanol: Glacial Acetic Acid (4:6:2:0.5 v/v/v/v). **Detection:** 255 nm **Linearity:** 1-35 ng/band and 6-21 ng/band for Aceclofenac and THC, respectively. **Correlation Coefficient:** 0.998 and 0.999 for Aceclofenac and THC, respectively. **R_f:** 0.79 and 0.16 for Aceclofenac and THC, respectively
26.	Forced degradation study of Thiocolchicoside: Characterization of Its	**Method: LC- MS** **Characterization By**: MS, NMR, IR

	degradation products	
Spectroscopic and Chromatographic Methods		
	In combination with other Drugs	
27.	Simultaneous estimation of Thiocolchicoside and Aceclofenac in pharmaceutical dosage form by Spectrophotometric and LC method	**UV Spectrophotometry** **Method A-** Area under Curve Method **Detection:** 264.5-254.5 nm, 249-259 nm and 279-269 nm for THC and Aceclofenac, respectively. **Linearity**: 4-36 ppm for both **HPLC** **Stationary Phase:** C_{18} column (4.6 mm x 250 mm, 5 µm). **Mobile Phase:** Acetonitrile: Water: 0.025M Potassium Dihydrogen Orthophosphate buffer ($_{P}$H 3) (70:10:20 v/v/v). **Flow rate :** 1.0 ml/min **Detection:** 260 nm **Retention Time:** 2.70min and 4.76 min THC and Aceclofenac, respectively.

3. AIM OF WORK:

Thiocolchicoside is a semi-synthetic sulphur derivative of colchicoside and used clinically for its anti-inflammatory, analgesic and muscle relaxant properties. Aceclofenac, Diclofenac sodium and Lornoxicam are the non-steroidal anti-inflammatory drugs used in inflammatory conditions.

Stability is an essential factor for quality, safety and efficacy of a drug product. A drug product, which is not stable, can result in changes in physical (hardness, dissolution rate, phase separation etc.) as well as chemical characteristics (formation of high risk decomposition substances). Degradation study of drug itself and its pharmaceutical formulation allows a better knowledge of its therapeutic, physicochemical and toxicological behaviour. The study of drug degradation kinetics is of greater importance for development of stable formulation and establishment of expiration date for commercially available drug products and also helps in deciding the routes of administration and storage conditions of various pharmaceutical dosage forms.

Literature describes degradation studies of thiocolchicoside in alkaline, acidic, oxidative and photolytic conditions. However, there was no published report found which describing the stability indicating HPTLC method for estimation of thiocolchicoside and its simultaneous estimation with anti-inflammatory drugs like aceclofenac, lornoxicam etc. in their pharmaceutical dosage forms and degradation kinetic study of thiocolchicoside in acidic, alkaline and oxidative conditions.

Therefore, it was thought of interest to develop a simple, accurate, precise and specific stability indicating HPTLC method for estimation of thiocolchicoside in pharmaceutical dosage forms and combined pharmaceutical dosage forms with anti-inflammatory drugs.
Hence the objectives of present work were

1. To develop and validate stability indicating HPTLC method for estimation of thiocolchicoside in its pharmaceutical dosage forms
2. Degradation kinetic study of thiocolchicoside in alkaline, acidic and oxidative medium.
3. Isolation and characterization of degradation products of thiocolchicoside formed in acidic and alkaline condition.

4. To develop and validate stability indicating HPTLC method for simultaneous estimation of thiocolchicoside and diclofenac sodium in their combined pharmaceutical dosage forms.
5. To develop and validate stability indicating HPTLC method for simultaneous estimation of thiocolchicoside and aceclofenac in their combined pharmaceutical dosage forms.
6. To develop and validate stability indicating HPTLC method for simultaneous estimation of thiocolchicoside and lornoxicam in their combined pharmaceutical dosage forms.

4. EXPERIMENTAL:

4.1 Development and validation of stability indicating high performance thin layer chromatography method for estimation of thiocolchicoside in pharmaceutical dosage form

4.1.1 Instrumentation:

→ Semi-automatic sample applicator (Camag Linomat V, Muttenz, Switzerland)

→ Camag TLC scanner IV

→ Camag Twin trough developing chamber (20 x 20 cm, 10 x 10 cm)

→ Hamilton syringe (100 µl)

→ Camag Win-CATS software

→ Double beam UV-Visible spectrophotometer (Shimadzu, model 1800 having two matched quartz cells)

→ Bruker Alfa FTIR-ATR instrument, Equipped with OPUS software

→ Electronic analytical balance – AUX-220, Shimadzu

→ Ultrasonicator

→ Hot air oven

→ Thiele's tube apparatus

4.1.2 Apparatus:

→ Volumetric flask- 10, 25, 50, 100 ml-Borosilicate glass type – I

→ Pipettes- 1, 2, 5, 10 ml

→ Measuring cylinder- 100 ml

→ Beakers- 25, 250, 500 ml

4.1.3 Chemicals and Materials:

→ Toluene (AR grade) – s.d. Fine- Chem Limited, Mumbai, India.

→ Methanol (AR Grade) – s.d. Fine- Chem Limited, Mumbai, India.

→ Ethyl acetate (AR Grade) – s.d. Fine- Chem Limited, Mumbai, India.

→ Thiocolchicoside, Aceclofenac, Diclofenac sodium and Lornoxicam were provided as a gift sample from reputed pharmaceutical companies of India.

→ Sodium hydroxide pellets

→ Hydrochloric acid

→ Hydrogen Peroxide

→ Whatman filter paper No. 41

→ Pharmaceutical dosage forms were procured from a local market.

4.1.4 Preparation of Solutions:

Preparation of Working Standard Solution of THC:

Accurately weighed 10 mg of THC was weighed transferred in 10 ml volumetric flask, dissolved in small volume of methanol and then diluted up to mark with methanol. From the above solution, 0.5 ml was transferred in to 25 ml volumetric flask and diluted up to the mark with methanol to get final concentration 20 µg/ml.

Preparation of sample solution for forced degradation:

Forced degradation of thiocolchicoside was carried out in 1.0 N HCl at 80^0 C, 0.1 N NaOH at 80^0 C, 1.0 % H_2O_2 at 80^0 C, sunlight for 8 hours, hot air oven at 110^0C and distilled water at 80^0 C.

4.1.5 Mobile phase optimization:

The standard stock solution of THC and degraded drug solution were spotted separately on pre-coated silica gel aluminium plate by using glass capillary tube and allowed it to dry in hot air oven. The different mobile phases (10 ml) was taken in 100 ml glass beaker and allowed it to saturate for 30 minutes. After saturation, the spotted plate was developed in mobile phase about ¾ height of the plate. The plate was removed and allowed it to dry in hot air oven. Spots were observed in U.V cabinet for tailing, shape, separation etc.

4.1.6 Selection of Wavelength for detection:

The spots of thiocolchicoside standard and alkaline degraded solution of thiocolchicoside were scanned by TLC scanner IV for selection of wavelength for alkaline degradation kinetic study of thiocolchicoside.

4.1.7 Chromatographic conditions:

Samples were spotted on TLC plate 15 mm from the bottom edge by Linomat V semi-automatic spotter using following parameters: band width, 6 mm; track distance, 11.6 mm; application rate, 0.1µl/s. The TLC plate was developed in Camag twin trough chamber using toluene : acetone :

water (1.5:7.5:1.0, v/v/v) as mobile phase at temperature, 25 ± 2 ˚C; relative humidity, 35 ± 5 %; chamber saturation time, 30 min; migration distance, 75 mm. The TLC plate was scanned and analysed by TLC Scanner IV and Win CATS software using following parameters: slit dimension, 4 × 0.30 mm; scanning speed, 20 mm/sec; detection wavelength, 370 nm and 277nm (only for alkaline degradation kinetic study).

4.1.8 Analysis of forced degradation samples:

From forced degraded sample solutions, 10µl of each solution were applied on same TLC plate, developed, dried and analysed as described under chromatographic conditions.

4.1.9 Procedure for Calibration Curve:

From working standard solution of THC (20µg/ml) 5, 10, 15 20 and 25 µl were spotted on a TLC plate, developed, dried and analysed as described under chromatographic conditions. Calibration curve was constructed by plotting peak area of THC against respective THC concentration.

4.1.10 Method Validation:

The developed method was validated for specificity, linearity, precision, accuracy, LOD and LOQ as per ICH guideline.

4.1.11 Procedure for assay of marketed formulations:

The marketed dosage form of thiocolchicoside was analysed by developed method and percentage of labeled claim was determined.

4.2 Degradation kinetic study of thiocolchicoside in different conditions:

The degradation kinetic study of thiocolchicoside was carried out in different strength of sodium hydroxide, hydrochloric acid and hydrogen peroxide at different temperature up to 150 minutes. The sample was analysed as described in chromatography conditions. From the data of sample analysis, degradation rate constant, half-life and shelf life were determined for thiocolchicoside in different stress conditions.

4.3 Isolation and characterization of degradation products of thiocolchicoside formed in acidic and alkaline condition

Accurately weighted 500mg of thiocolchicoside was completely degraded in HCl and NaOH. The degradation products were separated by neutralization of completely degraded solution. The Mass spectrum, UV spectrum, NMR spectrum, IR spectrum and melting point of degradation products were recorded for identification and characterization of degradation products.

4.4 Development and validation of stability indicating high performance thin layer chromatography method for simultaneous estimation of thiocolchicoside and aceclofenac in their combined pharmaceutical dosage forms

4.4.1 Preparation of Solutions:

Preparation of mixed working standard solution of THC and ACF:

Aliquot 0.4 ml of THC standard stock solution (100µg/ml) and 0.4 ml of ACF standard stock solution (500µg/ml) were transferred and diluted up to mark with methanol in 10ml volumetric flask to get solution having strength 4µg/ml of THC and 20µg/ml of ACF.

Preparation of sample solutions for forced degradation study:

Forced degradation of thiocolchicoside and aceclofenac was carried out in 1.0 N HCl at 80^0C, 0.1 N NaOH at 80^0C, and distilled water at 80^0C.

4.4.2 Selection of wavelength for Detection:

The standard solutions of THC (25µg/ml) and ACF (25µg/ml) were scanned in the range of 200-400 nm against methanol as blank in UV visible spectrophotometer. An overlain UV spectrum of THC and ACF was obtained.

4.4.3 Chromatographic Conditions:

Samples were spotted on TLC plate 15 mm from the bottom edge by Linomat V semi-automatic spotter using following parameters: band width, 6 mm; track distance, 11.6 mm; application rate, 0.1µl/s. The TLC plate was developed in Camag twin trough chamber using toluene: ethyl acetate: methanol: glacial acetic acid (7.0 : 1.0 : 2.0 :0.3 v/v/v/v) as mobile phase at temperature, 25 ± 2 ˚C; relative humidity, 35 ± 5 %; chamber saturation time, 30 min; migration distance, 75 mm. The TLC plate was scanned and analysed by TLC Scanner IV and WinCATS software

using following parameters: slit dimension, 4 × 0.30 mm; scanning speed, 20 mm/sec; detection wavelength, 278 nm.

4.4.4 Procedure for analysis of forced degradation samples:

From each forced degraded solution, 10µl of was applied to TLC plate, developed, dried, scanned and analysed at 278nm as described in chromatographic condition.

4.4.5 Procedure for Calibration Curve:

From the mixed working standard solution of THC and ACF, 5µl, 10µl, 15µl, 20µl and 25µl were spotted on pre-coated TLC plate, developed, dried, scanned and analysed at 278 nm as described in chromatographic condition.

4.4.6 Method Validation:

The developed method was validated for specificity, linearity, precision, accuracy, LOD and LOQ as per ICH guideline.

4.4.7 Procedure assay of marketed formulations:

The combined marketed dosage form containing thiocolchicoside and aceclofenac was analysed by developed method and percentage of labeled claim was determined.

4.5 Development and validation of stability indicating high performance thin layer chromatography method for simultaneous estimation of thiocolchicoside and diclofenac sodium in their combined pharmaceutical dosage forms

4.5.1 Preparation of Solutions:

Preparation of mixed working standard solution of THC and DCF:

Aliquot of 0.4 ml from THC standard stock solution (100µg/ml) and 0.4 ml from DCF standard stock solution (500µg/ml) were transferred and diluted up to mark with methanol in 10ml volumetric flask to get solution having strength 4µg/ml of THC and 20µg/ml of DCF.

Preparation of samples for forced degradation study:

Forced degradation of thiocolchicoside and diclofenac sodium was carried out in 1.0 N HCl at 80^0C, 0.1 N NaOH at 80^0C, and distilled water at 80^0C.

4.5.2 Selection of wavelength for Detection:

The standard solutions of THC (25µg/ml) and DCF (25µg/ml) were scanned in the range of 200-400 nm against methanol as blank in UV visible spectrophotometer. An overlain UV spectrum of THC and DFC was obtained.

4.5.3 Chromatographic Conditions:

Samples were spotted on TLC plate 15 mm from the bottom edge by Linomat V semi-automatic spotter using following parameters: band width, 6 mm; track distance, 11.6 mm; application rate, 0.1µl/s. The TLC plate was developed in Camag twin trough chamber using toluene: methyl acetate: methanol: glacial acetic acid (7.0:1.0:2.0: 0.3,v/v/v/v) as mobile phase at temperature, 25 ± 2 ˚C; relative humidity, 35 ± 5 %; chamber saturation time, 30 min; migration distance, 75 mm. The TLC plate was scanned and analysed by TLC Scanner IV and WinCATS software using following parameters: slit dimension, 4 × 0.30 mm; scanning speed, 20 mm/sec; detection wavelength, 278 nm.

4.5.4 Procedure for analysis of forced degradation samples:

From each forced degraded solution, 10µl of was applied to TLC plate, developed, dried, scanned and analysed at 278nm as described in chromatographic condition.

4.5.5 Procedure for Calibration Curve:

From the mixed working standard solution of THC and DCF, 5µl, 10µl, 15µl, 20µl and 25µl were spotted on pre-coated TLC plate developed, dried, scanned and analysed at 278 nm as described in chromatographic condition.

4.5.6 Method Validation:

The developed method was validated for specificity, linearity, precision, accuracy, LOD and LOQ as per ICH guideline.

4.5.7 Procedure for assay of marketed formulations:

The combined marketed dosage form containing thiocolchicoside and diclofenac sodium was analysed by developed method and percentage of labeled claim was determined.

4.6 Development and validation of stability indicating high performance thin layer chromatography method for simultaneous estimation of thiocolchicoside and lornoxicam in their combined pharmaceutical dosage forms

4.6.1 Preparation of Solutions:

Preparation of mixed working standard solution of THC and LNX:

Aliquot of 0.4 ml from THC standard stock solution (100µg/ml) and 0.4 ml from LNX standard stock solution (100µg/ml) were transferred and diluted up to mark with methanol in 10ml volumetric flask to get solution having strength 4µg/ml of THC and 4µg/ml of LNX.

Preparation of samples for forced degradation:

Forced degradation of thiocolchicoside and lornoxicam was carried out in 1.0 N HCl at 80^0C, 0.1 N NaOH at 80^0C, and distilled water at 80^0C.

4.6.2 Selection of wavelength for Detection:

The standard solutions of THC (25µg/ml) and LNX (25µg/ml) were scanned in the range of 200-400 nm against methanol as blank in UV visible spectrophotometer. An overlain UV spectrum of THC and LNX was obtained.

4.6.3 Chromatographic Conditions:

Samples were spotted on TLC plate 15 mm from the bottom edge by Linomat V semi-automatic spotter using following parameters: band width, 6 mm; track distance, 11.6 mm; application rate, 0.1µl/s. The TLC plate was developed in Camag twin trough chamber using toluene: dichloromethane: methanol: triethyl amine (6.0 : 2.0 : 2.0 :0.3 v/v/v/v) as mobile phase at temperature, 25 ± 2 ˚C; relative humidity, 35 ± 5 %; chamber saturation time, 30 min; migration distance, 75 mm. The TLC plate was scanned and analysed by TLC Scanner IV and WinCATS software using following parameters: slit dimension, 4 × 0.30 mm; scanning speed, 20 mm/sec; detection wavelength, 370 nm.

4.6.4 Procedure for analysis of forced degradation samples:

From each forced degraded solution, 10µl was applied to TLC plate, developed, dried, scanned and analysed at 278nm as described in chromatographic condition.

4.6.5 Procedure for Calibration Curve:

From the mixed working standard solution of THC and LNX, 5µl, 10µl, 15µl, 20µl and 25µl were spotted on pre-coated TLC plate, developed, dried, scanned and analysed at 370 nm as described in chromatographic condition.

4.6.6 Method Validation:

The developed method was validated for specificity, linearity, precision, accuracy, LOD and LOQ as per ICH guideline.

4.6.7 Procedure for assay of marketed formulations:

The combined marketed dosage form containing thiocolchicoside and lornoxicam was analysed by developed method and percentage of labeled claim was determined.

5. RESULTS AND DISCUSSION:

5.1 Development and validation of stability indicating high performance thin layer chromatography method for estimation of thiocolchicoside in pharmaceutical dosage form

5.1.1 Selection of wavelength for detection and mobile phase optimization:

The maximum wavelength of thiocolchicoside was selected for detection of thiocolchicoside as per IP 2010. The overlain spectra of standard THC and alkaline degraded product of THC showed that absorbance of standard THC and alkaline degraded product of THC was found to be suitable at 277nm. So, 277 nm was selected as the wavelength for detection for alkaline degradation kinetic study of thiocolchicoside. Thiocolchicoside and degraded sample solutions in different condition were spotted on the TLC plates and were run in different solvent systems. The mobile phase toluene: acetone: water (1.5:7.5:1.0 v/v/v) gave good resolution and compact spots with R_f value of 0.53 for THC.

5.1.2 Analysis of forced degradation samples of thiocolchicoside:

The chromatogram of acid treated THC was showed two additional peaks, the chromatogram of alkaline treated THC was showed two additional peaks, the chromatogram of hydrogen peroxide treated THC was showed three additional peak, the chromatogram of distilled water degraded THC was showed one additional peak, the chromatogram of photolytic degraded sample was showed one additional peak, and the chromatogram of dry heat degraded sample was showed one additional peak as compared to standard THC chromatogram.

5.1.3 Method validation:

Calibration curve of THC was found to be linear in the range of 100-500ng/spot at 370nm and 277nm. The 3D chromatogram of THC (100-500 ng/spot) at 370nm and 277nm were shown in fig. - 1 and 2 respectively.

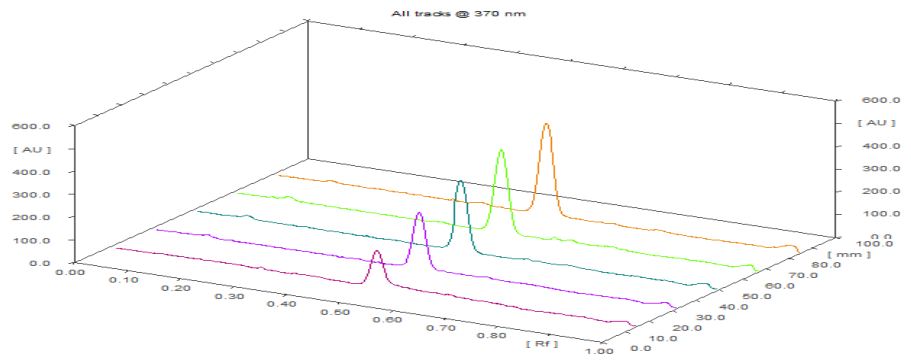


Figure-1: 3D chromatogram of THC (100-500ng/spot) showing Linearity at 370nm

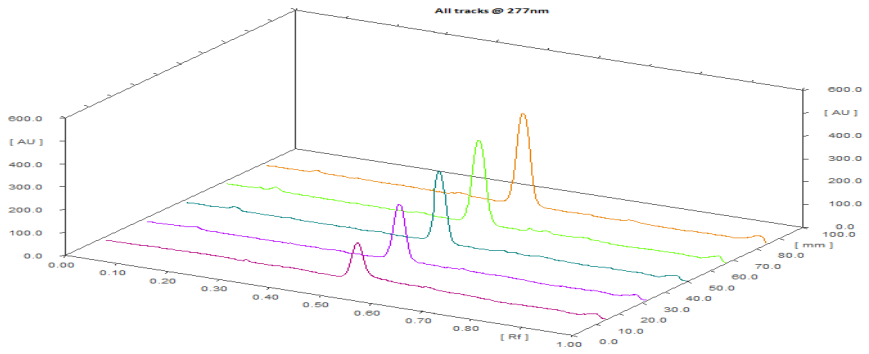


Figure-2: 3D Calibration curve of Thiocolchicoside (100-500ng/spot) at 277nm.

The % C.V. of repeatability, intra-day precision and inter-day precision were found to be less than two percentages for THC at both selected wavelength. The % recovery at each level was found to be within range of 98 to 102% for THC at both selected wavelength.

5.1.4 Assay of market formulations of thiocolchicoside:

The proposed method was applied for assay of capsule containing thiocolchicoside and the assay values were found to be 99.89 – 100.45% and 99.23 – 101.12% of labeled claim of thiocolchicoside at 370nm and 277nm respectively.

5.2 Degradation kinetic study of thiocolchicoside in alkaline medium:

The degradation rate constant and half-life for alkaline degradation of thiocolchicoside were found to be highest in 1.0 N NaOH and 60^0 C temperature. The degradation rate constant and half-life for acidic degradation of thiocolchicoside were found to be highest in 3.0 HCl and 80^0 C

temperature. The degradation rate constant and half-life for oxidative degradation of thiocolchicoside were found to be highest in 1.5 % H_2O_2 and 60^0 C temperature.

5.3 Isolation and characterization of degradation products of thiocolchicoside formed in acidic and alkaline condition

The THC standard was spotted on TLC plate with acidic and alkaline degradation products of THC and developed in toluene: acetone: water (1.5:7.5:1.0 v/v/v) in TLC chamber which is previously saturated with same mobile phase. The TLC plate was observed under UV light in UV cabinet. The photograph of observed THC plate was shown in figure-3. The spot of acidic (DP2) and alkaline degradation (DP1) were found to be at Rf 0.65 and the spot of acidic degradation product was found to be at Rf 0.80. The Rf of alkaline degradation product (DP1) and acidic degradation product (DP2) was found to be same. Thus one degradation product of acidic and alkaline degradation of THC was found to be identical.

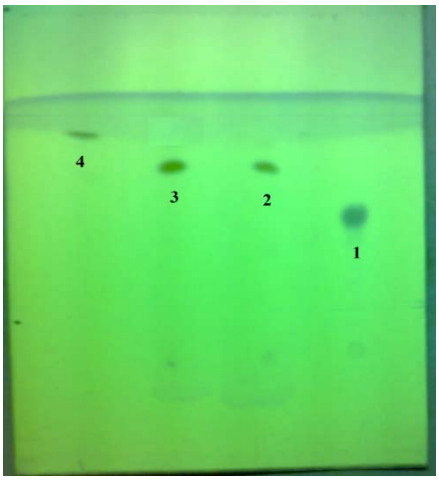


Figure-3: TLC plate showing standard THC (spot-1), alkaline degradation product DP1 (spot-2), acidic degradation product DP2 (spot-3), acidic degradation product DP3 (spot-4)

5.4 Development and validation of stability indicating high performance thin layer chromatography method for simultaneous estimation of thiocolchicoside and aceclofenac in their combined pharmaceutical dosage forms

5.4.1 Selection of wavelength for detection and mobile phase optimization:

The overlain UV spectrum of THC and ACF was showed suitable absorbance at 278 nm. Therefore, 278 nm was selected as detection wavelength for simultaneous estimation of both drugs. The mobile phase Toluene: Ethyl acetate: Methanol: Glacial acetic acid 7.0: 1.0:2.0: 0.3 (v/v/v/v) gave good resolution with Rf values 0.16±0.02 and 0.60±0.02 for THC and ACF respectively.

5.4.2 Analysis of forced degradation samples:

The chromatogram of acid treated mixture was showed two additional peaks of degradation products for both thiocolchicoside and aceclofenac respectively, the chromatogram of alkali treated mixture was showed two additional peaks of degradation products for both thiocolchicoside and aceclofenac respectively, the chromatogram of water treated mixture was showed one additional peak of degradation product for both thiocolchicoside and aceclofenac respectively as compared to standard chromatogram of both drugs.

5.4.3 Method validation:

Calibration curve was found to be linear in the range of 20 – 100 ng/spot for THC and 100 – 500 ng/spot for ACF. The 3D chromatogram of THC (20-100 ng/spot) and ACF (100-200 ng/spot) was showed figure-4.

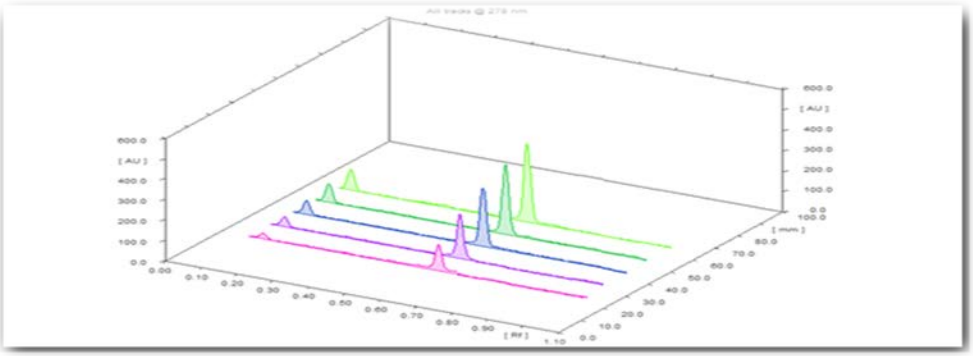


Figure-4: 3D chromatogram of THC (20-100 ng/spot) and ACF (100-500 ng/spot)

The % C.V. of repeatability, intra-day precision and inter-day precision were found to be less than two percentages for THC and ACF respectively. The % recovery at each level was found to be within range of 98 to 102% for THC and ACF respectively.

5.4.4 Assay of combined marketed formulations:

The assay values were found to be 99.12 – 100.32 and 99.55 – 99.76 of labeled claim of THC and ACF respectively.

5.5 Development and validation of stability indicating high performance thin layer chromatography method for simultaneous estimation of thiocolchicoside and diclofenac sodium in their combined pharmaceutical dosage forms

5.5.1 Selection of wavelength for detection and mobile phase optimization:

The overlain UV spectra of THC and DCF were showed appropriate absorbance at 278 nm. Therefore, 278 nm was selected as detection wavelength for simultaneous estimation of both drugs. The mobile phase Toluene: Methyl acetate: Methanol: Glacial acetic acid 7.0: 1.0:2.0: 0.3 (v/v/v/v) gave good resolution with Rf values 0.11±0.02 and 0.79±0.02 for THC and DCF.

5.5.2 Analysis of forced degradation samples:

The chromatogram of acid treated mixture was showed two additional peaks of degradation products for both thiocolchicoside and diclofenac sodium respectively, the chromatogram of alkali treated mixture was showed two additional peaks of degradation products for thiocolchicoside and one additional peak of degradation product for diclofenac sodium, the chromatogram of neutral condition treated mixture was showed two additional peaks of degradation product for thiocolchicoside and no additional peak of degradation product for diclofenac sodium as compared to standard chromatogram of both drugs.

5.5.3 Method validation:

Calibration curve was found to be linear in the range of 20 – 100 ng/spot for THC and 100 – 500 ng/spot for DCF. The 3D chromatogram of THC (20-100 ng/spot) and DCF (100-200 ng/spot) was showed in figure-5.

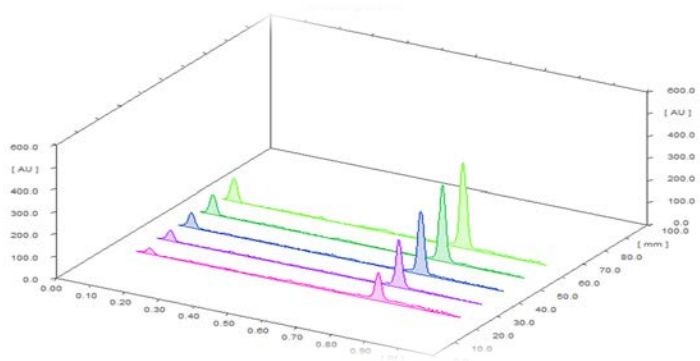


Figure-5: 3D chromatogram of THC (20-100 ng/spot) and DCF (100-500 ng/spot)

The % C.V. of repeatability, intra-day precision and inter-day precision were found to be less than two percentages for THC and DCF respectively. The % recovery at each level was found to be within range of 98 to 102% for THC and DCF respectively.

5.4.4 Assay of combined marketed formulations:

The assay values were found to be 100.32 – 101.61 and 98.68 – 99.78 of labeled claim of THC and DCF respectively.

5.6 Development and validation of stability indicating high performance thin layer chromatography method for simultaneous estimation of thiocolchicoside and lornoxicam in their combined pharmaceutical dosage forms

5.6.1 Selection of wavelength for detection and mobile phase optimization:

The overlain UV spectra of THC and LNX were showed suitable absorbance at 370 nm. Therefore, 370 nm was selected as detection wavelength for simultaneous estimation of both drugs. The mobile phase Toluene: Dichloromethane: Methanol: Triethyl amine 6.0: 2.0:2.0: 0.3 (v/v/v/v) gave good resolution with Rf values 0.25±0.02 and 0.60±0.02 for THC and LNX.

5.6.2 Analysis of forced degradation samples:

The chromatogram of acid treated mixture was showed two additional peaks of degradation products for both thiocolchicoside and Lornoxicam respectively, the chromatogram of alkali treated mixture was showed two additional peaks of degradation products for thiocolchicoside and one additional peak of degradation product for lornoxicam, the chromatogram of neutral

condition treated mixture was showed one additional peak of degradation product for thiocolchicoside and no additional peak of degradation product for Lornoxicam as compared to standard chromatogram of both standard drugs.

5.6.3 Method validation:

Calibration curve was found to be linear in the range of 20 – 100 ng/spot for THC and 20-100 ng/spot for LNX. The 3D chromatogram of THC (20-100 ng/spot) and LNX (20-100 ng/spot) was showed in figure-6.

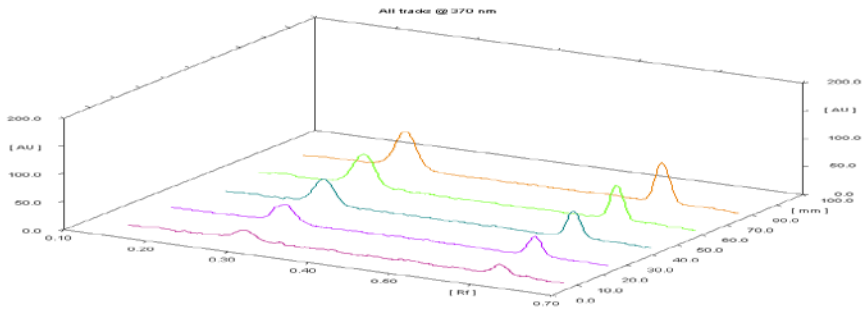


Figure-6: 3D chromatogram of THC (20-100 ng/spot) and LNX (20-100 ng/spot)

The % C.V. of repeatability, intra-day precision and inter-day precision were found to be less than two percentages for THC and LNX respectively. The % recovery at each level was found to be within range of 98 to 102% for THC and LNX respectively.

5.5.4 Assay of THC and LNX in combined marketed formulations:

The assay values were found to be 100.05 – 100.50 and 99.01 – 102.01 of labeled claim of THC and LNX respectively.

6. CONCLUSION:

Simple, specific, precise and accurate stability indicating HPTLC method for estimation of thiocolchicoside in its pharmaceutical dosage form has been developed and validated as per ICH guideline. The developed was extended for the degradation kinetic study of thiocolchicoside in alkaline, acidic and oxidative conditions. All degradations of thiocolchicoside follow first order kinetics. Degradation rate of thiocolchicoside increases as either strength of medium or temperature or both increases. The specific, precise and accurate stability indicating HPTLC methods for simultaneous estimation of thiocolchicoside and anti- inflammatory drugs like aceclofenac, diclofenac sodium and lornoxicam have been developed and validated as per ICH guideline. The proposed methods have been applied for simultaneous estimation of thiocolchicoside and anti-inflammatory drugs like aceclofenac, diclofenac sodium and lornoxicam in their combined pharmaceutical dosage forms.

7. REFERENCES:

1. Bennett PN, Brown MJ. Clinical Pharmacology. Tenth edition, 2008, 279-281.
2. Gilman AG, Hardman JG, Limbird LE. Goodman & Gilman's the pharmacological basis of therapeutics. Tenth edition, International edition, McGraw-Hill publisher's Medical publishing division, Newyork, 2001, 1181-1183.
3. Tripathi KD, Essentials of Medical Pharmacology, Fifth edition, Jaypee Brothers Medical Publishers, New Delhi, 2004, 754.
4. Cartenson JT, Swarbirck J. Solution kinetics- Drug stability principles and practice. Second edition, Marcel Dekker series, 1995, 17-19.
5. Sinko PJ, Martin's Physical Pharmacy and Pharmaceutical Sciences. Fifth edition, Indian edition, B. I. Publishers, Lippincott Williams and Wilkins A Wolters Kluwer Company, 2006, 397-436.
6. Maryadele J, Neil O, The Merck Index- An encyclopedia of chemicals, drugs and biological. Fourteenth ed. USA: Merck Research Laboratories; 2006. pp. 9429.
7. http//www.drugdatabank.com/Thiocolchicoside, Aceclofenac, Diclofenac sodium, Lornoxicam.
8. Government of India Ministry of Health and Family Welfare. Indian Pharmacopoeia, Vol 3. 6th ed. Ghaziabad, India: The Indian Pharmacopoeia Commission; 2010. pp. 2213-14.
9. Acharjya S, Mallick P, Panda P, Mathrusri MA. Spectrophotometric Methods for the Determination of Thiocolchicoside in Bulk and Pharmaceutical Dosage Forms**.** Journal of Pharma Education.2010; 1 (1):51-57.
10. Bandari A, Nawal M, Jathalia R, Bhandari M, Solanki R. UV Specrophotometric Determination of Thiocolchicoside from capsule dosage form. Journal of Pharmacy Research.2011; 4(12):4685-4687.
11. Wankhede SB, Zambare SS, Chitlange SS. Estimation of Thiocolchicoside and Ketoprofen in pharmaceutical dosage form by Spectrophotometric methods. Journal of Pharmacy Research 2010; 3 (4):707-710.
12. Thankappan S, Parmar A, Sailor B, Vekariya K, Shah D. simultaneous estimation of Etodolac and Thiocolchicoside by UV Spectrophotometric methods in tablet formulation. International Journal of Pharmaceutical innovations.2012; 2(2):192-200.

13. Umakar RA, Rewatkar NS, Charde MS, Charde RM. Simultaneous Estimation of Thiocolchicoside and Diclofenac potassium by UV Spectrophotometer using multicomponent method. International Journal of chemtech research.2011; 3(2):944-47.
14. Joshi RR, Gupta KR. Simultaneous UV Spectrophotometric determination of Thiocolchicoside and Diclofenac in pharmaceutical formulation. Pelagia Research library.2010; 1(2):44-51.
15. Acharjya SK, Rajesh Y, Panda P, Priyambada M&M, Annapurna M. Spectrophotometric methods for simultaneous estimation of Etoricoxib and Thiocolchicoside in bulk and combined pharmaceutical dosage form. Journal of Pharma Education.2010; 1(1):51-57.
16. Chaudhari BG, Trivedi JB. Simultaneous Spectrophotometry estimation of Thiocolchicosode and Dexketoprofen Trometamol in Pharmaceutical Dosage form. International Journal of Biomedical and Advance Research 2012; 3 (3):179-183.
17. Harde MT, Jadav SB, Dharma DL, Chaudhari PD. Development and validation of UV-Visible spectrophotometric methods for simultaneous estimation of Thiocolchicoside and Dexketoprofen in bulk and tablet dosage form. International journal of pharmaceutical sciences and drug research.2012; 4(2):160-163.
18. Tank PK, Shah RR, Shukala MH, Nayak PP, Patel AP. Development and validation of first order derivative spectroscopic estimation of Thiocolchicoside aand Dexketoprofen Trometamol in pharmaceutical dosage form. Pharma science monitor. An international Journal of pharmaceutical science.2012:2612-2622.
19. Nikhade RD, Thakur AD, Chaudhari SB, Chandewar AV. Simultaneous estimation of Paracetamol, Thiocolchicoside and Aceclofenac by UV spectrophotometer using multicomponent method. Journal of pharmacy research.2011;4(7):2297-2299.
20. Hapse SA, Thorave RR, Kadaskar PT, Shirsath AS, Dokhe MD. Novel spectrophotometric methods for simultaneous estimation and validation of Paracetamol, Thiocolchicoside and Aceclofenac in tablet dosage form by UV spectrophotometer. Journal of pharmacy research.2011;4(11):3928-3929.
21. Umarkar AR, Rewatkar NS, Chaple DR. Stability Indicating RP-HPLC method for estimation of Thiocolchicoside in Capsule Dosage Forms. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2011;2(1):750-756.

22. Joshi RR, Gupta KR, Jinnawar KS, Wadidkar SG. Development and validation of Stability indicating RP-HPLC and assay method for determination of Thiocolchicoside in capsule. American journal of Pharmtech research.2012;2(1):589-602.
23. Rathod K, Patel J. Simultaneous estimation of Etodolac and Thiocolchicoside in their combined marketed formulation by RP-HPLC. International journal of Pharm tech research.2012; 4(4):1513-1519.
24. Jadhav SD, Butle SR, Patil SD, Jagtap PK. Validated stability indicating RP-HPLC method for simultaneous determination and in vitro dissolution studies of thiocolchicoside and diclofenac potassium from tablet dosage form. Arabian Journal of Chemistry 2011; 67(6):829-832.
25. Marolia BP, Vanpariya DJ, Satani BH, Prajapati PB, Shah SA, Shah DR. Application of RP-HPLC method for simultaneous estimation of Thiocolchicoside and Diclofenac in commercially available capsules. American Journal of Pharmtech research.2012; 2(3):806-818.
26. Wankhed SB, Zambare SS, Dixit NR, Chitlange SS. RP-HPLC method for simultaneous estimation of Thiocolchicoside and Ketoprofen in combined dosage forms. Scholar Research Library.2010; 2(3):315-320.
27. Kumar S, Joshi A, Thakar R, Pathak AK, Shah K. Simultaneous estimation of Etoricoxib and Thiocolchicoside by HPLC method in combined dosage forms. Acta Poloniae Pharmaceutical Drug Research.2011; 68(6):839-845.
28. Harde MT, Dharma DL, Jadav SB, Balap AR. Development and validation of RP-HPLC method for the simultaneous estimation of Thiocolchicoside and Dexketoprofen in bulk and pharmaceutical dosage forms. International Journal of Pharm Tech Research.2012; 4(4):1797-1802.
29. Bhavsar SM, Patel DM, Khandhar AP, Patel CN. Validated RP-HPLC method for simultaneous estimation of Lornoxicam and Thiocolchicoside in solid dosage form. Journal of Chemical and Pharmaceutical Research 2010: 2(2): 563-572.
30. Dhaneshvar SR, Raut KO, Bhusari VK. Validated HPLC method for simultaneous estimation of Peracetamol, Aceclofenac, and Thiocolchicoside in bulk drug and formulation. Research journal of Pharmaceutical Biological and Chemical science.2011; 2(2):435-445.

31. Shaoo M, Syal P, Hable AA, Raut RP, Chaudhari VP, Kuchekar BS. Development and validation of HPTLC method for simultaneous estimation of Lornoxicam and Thiocolchicoside in combined dosage form. Pharmaceutical Methods.2011; 2(3):178-183.
32. Patil ST, Bhusari VK, Dhaneshwar SR. Validated HPTLC method for simultaneous estimation of Thiocolchicoside and Aceclofenac in bulk drug and formulation. International Journal of Pharma and Bio Sciences 2011; 2(2): 0975-6299.
33. Del GE, Aprile S, Grosa G. Forced degradation study of thiocolchicoside: Characterization of its degradation products. Journal of Pharmaceutical and Biomedical Analysis 2012; 61:215–223.
34. Chitlange SS, Shinde PS, Pawbake GR, Wankhede SB. Simultaneous estimation of Thiocolchicoside and Aceclofenac in pharmaceutical dosage form by spectrophotometric and LC method. Scholars Research Library Der Pharmacia Letter 2010; 2(2): 86-93.

Submitted by	**Forwarded by**
Mr. Pintu B. Prajapati **Ph.D. Student**	**Dr. Shailesh A. Shah** **Supervising teacher for Ph. D. degree**